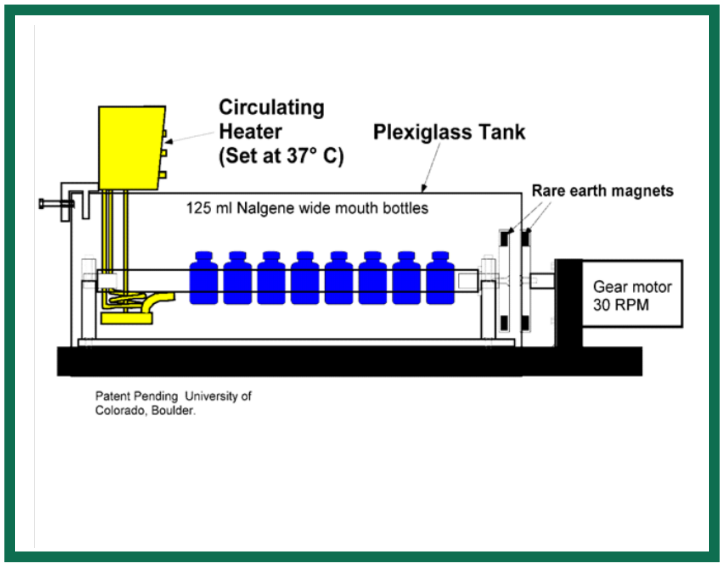
ESTCP
Cost and Performance Report

(ER-200916)



Validation of an In Vitro Bioaccessability Test Method for the Estimation of the Bioavailability of Arsenic from Soil and Sediment

December 2012

ESTCP

ENVIRONMENTAL SECURITY
TECHNOLOGY CERTIFICATION PROGRAM

U.S. Department of Defense

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
DEC 2012	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Validation of an In Vitro Bioaccessability Test Method for the Estimation of the Bioavailability of Arsenic from Soil and Sediment	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **ESTCP Office 4800 Mark Center Drive Suite 17D08 Alexandria, VA 22350-3605**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**SAR**	**34**	

COST & PERFORMANCE REPORT
Project: ER-200916

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

ACRONYMS AND ABBREVIATIONS

ºC	degrees Celsius
µg/g	micrograms per gram
µg/L	micrograms per liter
µm	micromoles
AFB	Air Force Base
ATSDR	Agency for Toxic Substances and Disease Registry
CBR	CB Research International
CSF	cancer slope factor
EPA	U.S. Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
FeOOH	iron oxide/hydroxide
g	grams
HDPE	high-density polyethylene
IRIS	Integrated Risk Information System
IVBA	*in vitro* bioaccessibility
IVIVC	*in vivo*-in vitro correlation
L	liters
M	molar
mg/kg	milligrams per kilogram
mL	milliliter
OU1	Operable Unit 1
QC	quality control
R^2	coefficient of determination
RBA	relative bioavailability
RfD	reference dose
RPM	revolutions per minute
SOP	standard operating procedure
TRW	Technical Review Workgroup

ACKNOWLEDGEMENTS

The work described in the report was accomplished through the efforts of a team of scientists. The co-investigators for this project were Susan Griffin of the U.S. Environmental Protection Agency, and Yvette Lowney of Exponent, Inc. The co-investigators were supported throughout the study by John Drexler of the University of Colorado at Boulder, who performed all of the *in vitro* bioaccessibility and speciation analyses, and also provided many valuable discussions and insights. In addition, the project was supported by scientists from SRC, Inc. (William Brattin, Gary Diamond, and Penny Hunter) and from CDM Smith (Lynn Woodbury), who provided ongoing support in data reduction and modeling efforts, as well as authorship of project reports.

This page left blank intentionally.

1.0 EXECUTIVE SUMMARY

1.1 OBJECTIVES OF THE DEMONSTRATION

Accurate evaluation of the human health risk from ingestion of arsenic in soil or soil-like media requires knowledge of the relative bioavailability (RBA) of arsenic in the soil or soil-like material. In general, studies to date have indicated that the RBA of arsenic in soil is lower than the U.S. Environmental Protection Agency (EPA) default value of 100%. Consequently, estimation of site-specific RBA values can often save substantial costs during site cleanup.

Although RBA can be measured using studies in animals, such studies are generally slow and costly. An alternative strategy is to perform measurements of arsenic solubility in the laboratory. Typically, a sample of soil or sediment is extracted using a fluid that has properties that resemble a gastrointestinal fluid, and the amount of arsenic solubilized from the sample into the fluid under a standard set of extraction conditions is measured. The fraction of arsenic that is solubilized is referred to as the *in vitro* bioaccessibility (IVBA). The IVBA is then utilized to predict the *in vivo* RBA of arsenic in that sample, usually through an empiric correlation model.

The objective of this demonstration project was to develop, optimize, and validate an IVBA-based method to accurately predict the RBA of arsenic in soil and soil-like materials.

1.2 TECHNOLOGY DESCRIPTION

The technology consists of two parts. In the first part, the IVBA of arsenic is measured. This is achieved by placing 1 gram (g) of test material in 100 milliliters (mL) of extraction fluid and extracting for 1 hour at 37 degrees Celsius (°C) with constant end-over-end mixing. A sample of the extraction fluid is removed and analyzed for arsenic. The IVBA value is calculated as the mass of arsenic solubilized in the fluid divided by the mass of arsenic contained in the sample extracted. In the second part, the RBA of arsenic is estimated from the IVBA value using an empirical mathematical model:

$$RBA = a + b\ IVBA$$

The parameters of the model (a and b) are derived by fitting the model to a "calibration" data set of test materials that have both a reliable *in vivo* RBA measurement and a reliable IVBA measurement.

1.3 DEMONSTRATION RESULTS

Test materials used to establish the correlation between IVBA and RBA included 20 materials where RBA had been measured in juvenile swine, and 17 samples where RBA had been measured in monkeys. Based on extensive and systematic investigation of a wide range of differing extraction conditions, it was found that no single method would yield high quality RBA predictions for the combined data set. However, each data set could be successfully modeled independently. For swine, the optimum extraction fluid is 0.4 molar (M) glycine at pH 1.5, and the best fit regression model is:

$$RBA(swine) = 19.7 + 0.622\ IVBA_{pH1.5} (R^2 = 0.723)$$

For monkey, the optimum extraction fluid is 0.4 M glycine plus 0.05 M phosphate at pH 7, and the best fit regression model is:

$$RBA(monkey) = 14.3 + 0.583\ IVBA_{pH7} (R^2 = 0.755)$$

The finding that the best-fit regression model occurs at pH 7 for monkey and pH 1.5 for swine suggests that there might be significant physiological differences between the animal species that result in this outcome. However, this study did not seek to investigate the reason why different extraction pH conditions yielded a better fit for swine and monkey, so no mechanistic explanation is available at this time.

The within- and between-laboratory precision of the IVBA method was evaluated by triplicate analysis of each of 12 soils for each of three extraction fluids by each of four laboratories. Within-laboratory precision was evaluated by examining the magnitude of the standard deviation for three replicate values for each of 12 test materials. Within-laboratory precision was typically less than 3%, with an average of 0.8% for all four laboratories. Between-laboratory precision was evaluated by examining the between-laboratory variability in the mean IVBA values for each test soil for each extraction condition. Between-laboratory variation in mean values was generally less than 7%, with an overall average of 3%. These results demonstrate the method is highly reproducible, both within and between laboratories.

The principal advantage of this IVBA-based method compared to measurement of RBA *in vivo* is that it is much less expensive and much more rapid. For example, a typical *in vivo* RBA study may cost up to $100,000 and require several months for assessment of two samples, while a typical IVBA study can perform 40-60 extractions in 1 day at a cost of about $100 per extraction. This has the additional advantage that multiple samples (20 or more) may be collected from a site to ensure a robust characterization of IVBA/RBA across the site.

The principle advantages of this IVBA method compared to other *in vitro* methods that have been described in the literature are that 1) the fluids and extraction conditions are simple; 2) the results have been calibrated against a larger data set than any other method; and 3) the method has been demonstrated to be reproducible both within and between laboratories.

1.4 IMPLEMENATION ISSUES

There are no significant issues associated with implementation of this technology. Several commercial and EPA laboratories currently provide IVBA extraction analyses. The method has been developed in close coordination with EPA's Bioavailability Subcommittee of the Technical Review Workgroup (TRW), and the method is expected to be acceptable to EPA for use in evaluating risks from arsenic at sites where soil, sediment, or other soil-like media contain elevated levels of arsenic.

2.0 INTRODUCTION

2.1 BACKGROUND

Arsenic in soil or other soil-like media may be a contaminant of potential human health concern at a variety of sites, epically those where mining, smelting, leather tanning, wood preservation, or pesticide manufacture and/or application has occurred.

Accurate assessment of the human health risks resulting from incidental ingestion of arsenic-containing soil requires knowledge of the amount of arsenic that is absorbed from the soil into the body. This is referred to as bioavailability. Absorption of arsenic following oral ingestion of contaminated soil or sediment depends mainly on the physical and chemical attributes of the arsenic in the soil. Some forms of arsenic (e.g., sodium arsenate) are readily soluble in gastrointestinal fluid and are well absorbed into the blood in most species (Juhasz et al., 2006; ATSDR, 2007). Other forms of arsenic (e.g., arsenic adsorbed to iron-containing particles in soil) that are not as readily dissolved are generally not as extensively absorbed. Because the form of arsenic in soil varies widely from site to site (depending mainly on source), the bioavailability of arsenic in soil also varies widely from site to site.

In general, it is most convenient and useful to measure the ratio of the bioavailability of arsenic in a site soil compared to an appropriate reference material (usually sodium arsenate). This is referred to as RBA. When a reliable RBA value is available for a particular site medium (e.g., soil), the RBA can be used to adjust the default oral reference dose (RfD) (RfD_{IRIS}[1]) and oral cancer slope factor (CSF) (CSF_{IRIS}) for arsenic to account for differences in absorption between arsenic ingested in water and arsenic ingested in the site medium.

In the absence of reliable site-specific data, the conservative default approach is to assume an RBA of 100% for arsenic in soil and sediment. However, studies performed to date indicate that this assumption is generally too high, with most measured RBA values ranging from 5% to 50% (Roberts et al., 2007; EPA, 2010). Hence, when site-specific arsenic RBA can be reliably measured, it often reduces the estimated health risk from arsenic in soil, and this in turn can result in substantial cost savings during site cleanup.

Arsenic RBA can be measured *in vivo* using animal models (e.g., swine, monkey, or mice), and this is the preferred strategy whenever feasible. However, the cost (up to $100,000) and time (up to 6 months) requirements of *in vivo* RBA tests often limit the application of these models to only the largest sites. Therefore, a faster, more economical yet dependable *in vitro* method for predicting *in vivo* RBA is highly desirable.

One such alternative strategy is to perform measurements of arsenic solubility in the laboratory. Typically, a sample of soil or sediment is extracted using a fluid that has properties that resemble a gastrointestinal fluid, and the amount of arsenic solubilized from the sample into the fluid under a standard set of extraction conditions is measured. The fraction of arsenic that is solubilized is referred to as the IVBA. The IVBA is then utilized to predict the *in vivo* RBA of arsenic in that sample, usually through an empiric correlation model.

[1] Integrated Risk Information System

2.2 OBJECTIVE OF THE DEMONSTRATION

The objective of this demonstration project was to develop, optimize, and validate an IVBA method to estimate RBA of arsenic from soil for use in human health risk assessments.

2.3 REGULATORY DRIVERS

EPA's Risk Assessment Guidance for Superfund Part A (EPA, 1989) and EPA's Guidance for Evaluating the Bioavailability of Metals in Soils for Use in Human Health Risk Assessment (EPA, 2007a) both indicate that it is acceptable and appropriate to make site-specific adjustments to exposure and risk estimates when reliable site-specific data are available to show that the absorption (bioavailability) of a contaminant from site media (e.g., soil or sediment) is different than the absorption of that chemical in studies used to derive the toxicity values.

As noted above, when these data are derived from reliable studies in an appropriate animal model, the data are generally considered to be acceptable. However, use of RBA values derived using an *in vitro* methodology requires that the *in vitro* test method meet a number of criteria, as detailed in EPA (2007b). The key requirements for IVBA-based technologies include the following:

- The method should have undergone independent scientific peer review.
- Data generated by the method should adequately measure or predict the toxic endpoint of interest (i.e., RBA measured in animals).
- The test method must be robust (relatively insensitive to minor changes in protocol) and transferable among properly equipped and staffed laboratories.
- The method should be time- and cost-effective.

The project reported here achieves these requirements and is expected to be acceptable to EPA for use in human health risk assessments of arsenic ingestion from soil or sediment.

3.0 TECHNOLOGY

3.1 TECHNOLOGY DESCRIPTION

The technology developed during this project consists of an extraction system to measure the IVBA of arsenic in a test material under specified conditions, coupled with a set of mathematical models to predict the RBA of the test material from the measured IVBA value.

3.1.1 Extraction System

Figure 1 illustrates the extraction device used in these studies. The device holds ten 125 mL wide-mouth high-density polyethylene (HDPE) bottles. These are rotated within a Plexiglas water tank maintained at 37 ± 2 ºC.

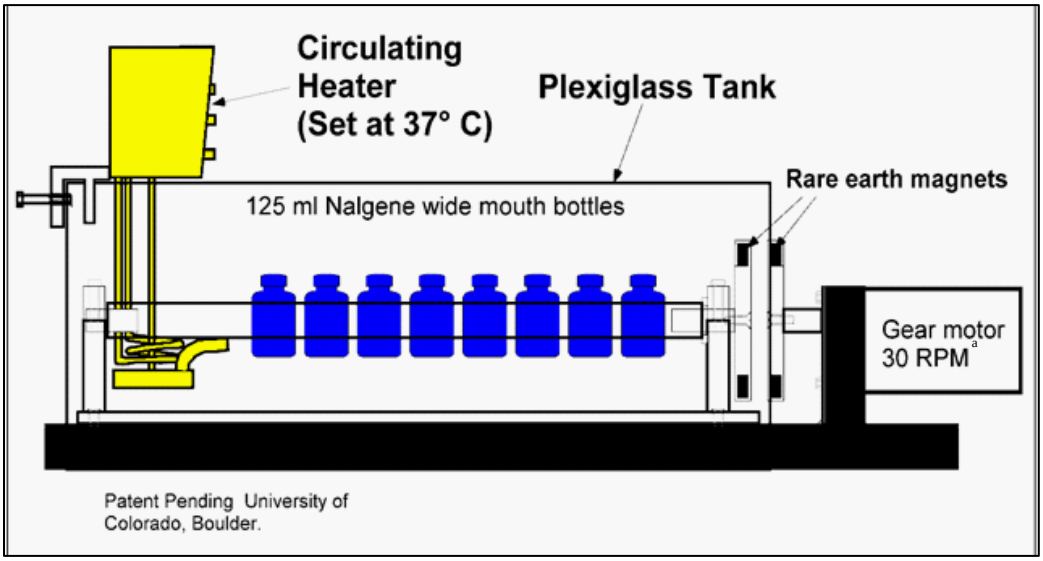


Figure 1. IVBA extraction device.

[a]revolutions per minute

To perform an IVBA extraction, one gram of test material is placed in an extraction bottle, and 100 mL of an appropriate extraction fluid is added. The composition of the extraction fluid that is generally recommended for use consists of 0.4 M glycine buffer adjusted to pH 1.5.

The bottles containing the test material and the extraction fluid are then placed into the extraction device and rotated end-over-end at 30 revolutions per minute for 1 hour. After 1 hour, the bottles are removed and a sample of the extraction fluid is withdrawn through a cellulose acetate disk filter for arsenic analysis using EPA Method 6020.

3.1.2 Calculation of IVBA

The IVBA of arsenic in the test material is calculated as follows:

$$IVBA = \frac{C_{fluid} \cdot V_{fluid}}{C_{soil} \cdot M_{soil}}$$

where

C_{fluid} = Concentration of arsenic in the extraction fluid (micrograms per liter [µg/L])
V_{fluid} = Volume of extraction fluid (liters [L])
C_{soil} = Concentration of arsenic in the test soil (micrograms per gram [µg/g]), measured using EPA Method 3050
M_{soil} = Mass of soil placed in the extraction bottle (g)

3.1.3 Calculation of RBA

The RBA of arsenic in the test material is estimated from the IVBA value using an equation of the following form:

$$RBA = a + b\,IVBA$$

The values of the model parameters (a and b) are derived empirically using regression analysis to fit the model to a calibration data set of samples for which reliable values of IVBA and *in vivo* RBA have both been measured. For a prediction of RBA (as a percentage) measured in swine, the best extraction fluid is 0.4 M glycine (pH 1.5), and the best fit prediction model is:

$$RBA_{swine}(\%) = 19.7 + 0.62 \cdot IVBA_{pH\ 1.5}$$

For a prediction of RBA measured in monkeys, the best extraction fluid is 0.4 M glycine and 0.05 M phosphate adjusted to pH 7, and the best fit prediction model is:

$$RBA_{monkey}(\%) = 14.3 + 0.58 \cdot IVBA_{pH\ 7}$$

The finding that the best-fit regression model occurs at pH 7 for monkey and pH 1.5 for swine suggests that there might be significant physiological differences between the animal species that result in this outcome. However, this study did not seek to investigate the reason why different extraction pH conditions yielded a better fit for swine and monkey, so no mechanistic explanation is available at this time.

3.2 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

3.2.1 Advantages and Limitations Compared to *In Vivo* RBA Measurements

As noted previously, *in vivo* measurements of arsenic RBA typically require up to 6 months to plan and complete and can cost up to $100,000. Because of this, *in vivo* methods are typically limited to assessing a small number of samples at a site (e.g., one to four). In contrast, the primary advantage of the IVBA approach described here is that it is rapid (40 or more samples per day) and inexpensive (typically about $50 to $100 per IVBA extraction). As a result, the *in vitro* IVBA methods can be applied to a large number of samples (e.g., 10-50), allowing a more robust characterization of arsenic RBA at a site.

The principal limitation of the *in vitro* method is that the RBA value predicted from an IVBA measurement may not be identical to the RBA value that would have been derived had an *in vivo* study been performed. Rather, the predicted RBA value is what would be typical for a sample with the measured IVBA. However, it is important to recognize that *in vivo* RBA values have measurement error which introduces uncertainty into the estimate of the RBA, and the prediction error from the IVBA approach is about the same magnitude as the measurement error in a typical *in vivo* RBA estimate. Also, in practice, the small number of soil samples usually assessed using *in vivo* methods introduces additional uncertainty in site-wide characterization of RBA because this small number of samples cannot allow assessment of variability in RBA across the site.

3.2.2 Advantages and Limitations Compared to Other *In Vitro* IVBA-Based Prediction Models

A number of other researchers have described *in vitro* systems for measuring the extractability of arsenic from soil or other soil-like materials (see Table 1). The principal advantages of the method described here compared to other published methods include the following:

- The current method utilizes a single extraction step. This is in contrast to methods that utilize two or more sequential extraction steps, each intended to represent differing parts of the gastrointestinal system.
- The current method utilizes simple extraction fluids. This is in contrast to methods that seek to create extraction fluids that closely mimic various gastrointestinal fluids, including the presence of a number of biochemical constituents such as enzymes and metabolites.
- The current method is based on a more extensive and systematic testing of extraction conditions to identify the optimal conditions that most other approaches.
- The current method utilizes a larger set of calibration samples to establish the *in vitro-in vivo* correlation (IVIVC) between IVBA and RBA than any other approach. Indeed, some methods provide no information on IVIVC. The use of a large calibration data set is important because finding a successful model for a small set of samples appears to be substantially easier than finding a model for a wide variety of samples.
- The current method has undergone inter-laboratory validation, while, to our knowledge, no other approaches have been subjected to true inter-laboratory validation.

In summary, the current method is distinguished primarily by its simplicity, reliability, and degree of validation.

Table 1. Overview of published IVBA procedures for arsenic.

Reference	Phases	Gastric Fluid pH	Gastric Extraction Time	Intestinal Fluid pH	Intestinal Extraction Time	Test Material: Extraction Fluid Ratio	Gastric Solution	Intestinal Solution	Method Complexity	IVIVC Calibration Soils	Round Robin Validation ?
Basta et al. (2007)	2 (stomach/ intestinal)	1.8	1.0	5.5	1.0	1:150	HCl, NaCl, pepsin	$NaHCO_3$ (Na_2CO_3), bile extract, pancreatin	Moderate	15	No
Bruce et al. (2007)	2 (stomach/ intestinal)	1.3	1.0	7.0	3.0	0.4:40	HCl, pepsin, sodium citrate, malic acid, lactic acid and acetic acid	Na_2HCO_3, bile extract, pancreatin	High	9	No
Buckley (1997)	5[a]	1.8	1.0	7.0	5.0	unknown	HCl, $CaCl_2$, KCl, NaCl, $MgCl_2$, $FeCl_3$, KI, $NaPO_4$	Na_2HCO_3, $KHCO_3$	High	None	No
CBR (1993)	2 (stomach/ intestinal)	2.0	1.0	6.9	1.5	1:0.03	HCl	Na_2HCO_3/NaOH,	High	None	No
Ellickson et al. (2001)	3 (saliva/stomach/ intestinal)	1.4	2.0	6.5	2.0	0.05:100	HCl, NaCl, pepsin	Na_2HCO_3	High	1	No
Juhasz et al. (2007)	1 (stomach)	1.5	1.0	--	--	1:100	HCl, Glycine	---	Low	12	No
Medlin (1997)	2 (stomach/ intestinal)	1.5	1.0	---	3.0	1:110	HCl, pepsin, citrate, malate, lactic and acetic acids	Na_2HCO_3, bile extract, pancreatin	High	6	No
Oomen et al. (2002)	1 (stomach)	1.5	1.0	---	---	1:100	HCl, glycine	---	Low	None	No
Oomen et al. (2002)	2 (stomach/ intestinal)	2.0	2.0	7.5	6.0	2:100	HCl, pepsin, mucin	Na_2HCO_3, trypsin, pancreatine, bile extract	High	None	No
Wragg et al. (2002)	3 (saliva/stomach/ intestinal)	1.2	1.0	6.3	4.0	0.6:13.5	HCl, pepsin, mucin, BSA	Na_2HCO_3, pancreatine, lipase, bovine serum albumin, bile extract	High	9-11	Partial
Oomen et al. (2002)	2 (stomach/ intestinal)	4.0	3.0	6.5	5.0	1:2.5	HCl, pepsin, mucin, cellobiose, proteose, peptone starch	Na_2HCO_3, pancreatine	High	None	No
Oomen et al. (2002)	5[a]	2.5	1.5	6.8	6.0	1:25	HCl, pepsin, lipase	Na_2HCO_3, pancreatine	High	None	No
Rodriguez et al. (1999)	2 (stomach/ intestinal)	1.8	1.0	5.5	1.0	1:150	HCl, NaCl, pepsin	Na_2HCO_3, bile extract, pancreatin	Moderate	15	No
Ruby et al. (1996)	2 (stomach/ intestinal)	2.5	1.0	7.0	3.0	1:100	HCl, pepsin, citrate, malate, lactic and acetic acids	Na_2HCO_3, bile extract, pancreatin	High	3	No

[a]Extensive extraction procedure, including saliva, esophagus, stomach, small and large intestine steps.

4.0 PERFORMANCE OBJECTIVES

Performance objectives are the primary criteria for evaluating the success or failure of a new technology. They provide the basis for evaluating the performance and costs of the technology. Meeting these performance objectives is essential for successful demonstration and validation of the technology.

Table 2 provides a summary of the performance objectives that were established at the outset of the project, along with a summary of the degree to which each objective was achieved. These are discussed briefly below.

4.1 PERFORMANCE OBJECTIVE 1: IDENTIFY PRINCIPAL VARIABLES AFFECTING IVBA RESULTS

Variables that were studied included pH of the extraction fluid, temperature of the bath, time of extraction, fluid ionic strength, oxyanion (phosphate) addition, hydroxylamine addition, filter pore size, redox potential, and soil mass. The effect of varying these parameters was evaluated individually, holding all other extraction conditions constant.

Although all of these variables had effects on the IVBA of at least some test materials, the three that were considered to be the strongest determinants of IVBA were pH, phosphate concentration, and hydroxylamine hydrochloride concentration. Some variables impacted the IVBA of nearly all test materials in a similar fashion, while others impacted some test soils more than others.

4.2 PERFORMANCE OBJECTIVE 2: IDENTIFY OPTIMIZED COMBINATIONS OF KEY VARIABLES

Data were collected for 16 different test soils under 21 different combinations of extraction pH, phosphate concentration, and hydroxylamine concentration (a total of 336 extractions).

The primary success criterion established for this phase was that one or more extraction conditions yielded a correlation coefficient of 0.8 or better. (Note: a correlation coefficient of 0.8 is equivalent to a linear regression coefficient of determination [R^2] value of 0.64). This criterion was achieved in 11 cases when the RBA data were measured in swine, in 14 cases when the RBA data were measured in monkey, and in 11 cases when the data sets were combined.

4.3 PERFORMANCE OBJECTIVE 3: DETERMINE A FINAL PROTOCOL AND TEST ON ALL SOILS

Based on the results above, three different extractions conditions were selected for evaluation using a set of 35 test materials. The extraction fluids tested included: 1) pH 1.5 (no additions), 2) pH 7 (no additions), and 3) pH 7 plus 0.05 M phosphate and 0.1 M hydroxylamine.

Table 2. Performance objectives.

Performance Objective	Data Requirements	Success Criteria	Results
1) Identify principal variables affecting assay results.	Test the effect of pH, temperature of the bath, time of extraction, fluid composition (ionic strength, competitive binding agents), and filter pore size on metal extraction in the IVBA system.	Either the comparison between *in vivo* RBA and the IVBA RBA yield a correlation coefficient of 0.8 or better using linear regression, or specific mineralogical forms can be identified which react differently to one or more variables of interest in the IVBA system.	Achieved: Key variables that impact IVBA are extraction fluid pH, presence/absence of phosphate, and presence/absence of other agents such as hydroxylamine.
2) Determine up to three combinations of assay variables that are most likely to improve the predictive relationship between IVBA and RBA.	Key variables of interest identified in the previous step will be evaluated in a Latin square design. Assay variables of combinations that yield the highest R^2 and, secondarily, the lowest intercept, would be selected for further evaluation in a more demanding optimization evaluation.	Either regression analysis relating *in vivo* RBA to IVBA yields a correlation coefficient of 0.8 or better, or specific mineralogical forms can be identified which yield a correlation coefficient of 0.8 or better when run in two or more combinations of optimized variables.	Achieved: Three extraction conditions that provide best correlation with in vivo results include: 1) pH 1.5 (no additions), 2) pH 7 (no additions), and 3) pH 7 with phosphate and hydroxylamine.
3) Determine final protocol and test on all soils.	Test materials will be assayed using up to three test protocols identified from the previous step. Multiple assessments of each test material set will be conducted to assess reproducibility of results from each protocol. Analyze data by a series of regression models for each test protocol relating IVBA and RBA for each test material.	A single protocol is generated or several protocols are generated specific to the mineralogical form of arsenic.	Achieved: For prediction of RBA measured in swine, best extraction condition is pH 1.5 (R^2 = 0.72). For RBA values measured in monkey, best extraction fluid is pH 7, with or without additions (R^2 = 0.71 to 0.75).
4) Quantify the intra- and inter-laboratory reproducibility of the optimized protocol.	In a round-robin analysis, three independent laboratories will test each of several soils following the new optimized IVBA protocol. The results will be input into a regression model that best describes the relationship between IVBA and RBA for that protocol. All of the data on within and between-laboratory variability, the between laboratory correlation results for IVBA and RBA, and the results of the QC[a] samples (blanks and duplicates) included in the analysis.	The initial acceptance criterion for precision will be defined as the high end of the precision achieved by the primary laboratory. Absolute percent error, root-mean percent error and percent predictive error will also be calculated to evaluate predictive performance following methods described in Malinowski et al. (1997). Acceptance criteria and control limits will be based on limits established by Drexler and Brattin (2007).	Achieved: Both within laboratory and between laboratory precision in measured IVBA values is high (variability is generally less than 10%).

[a]quality control

The data generated were evaluated by fitting regression models relating IVBA and RBA for each extraction condition. The success criterion was a correlation coefficient of 0.8 or higher (an R^2 value of 0.64 or higher). This objective was achieved using pH 1.5 IVBA data for the swine data set ($R^2 = 0.72$) and using pH 7 IVBA data for the monkey data set, either with phosphate and hydroxylamine ($R^2 = 0.75$) or without any other additions ($R^2 = 0.71$). No extraction condition was found that satisfied this criterion when the swine and monkey data were combined.

4.4 PERFORMANCE OBJECTIVE 4: EVALUATE INTER-LABORATORY REPRODUCIBILITY

A set of 12 test materials was distributed to each of four laboratories (including the University of Colorado reference laboratory), along with a standard operating procedure (SOP) detailing the proper technique for obtaining arsenic IVBA measurements. Each laboratory measured the IVBA of each material in triplicate, for each of three different extraction fluids (a total of 108 extractions per laboratory).

The success criterion for within-laboratory precision on IVBA measurements was defined as the high end of the precision achieved by the reference laboratory (University of Colorado, Boulder). This value is 6%. The results for the three round-robin laboratories were all within this value, and overall within-laboratory precision for each laboratory was similar to that of the reference laboratory.

No *a priori* criterion was established for between-laboratory precision, since this value is generally established empirically from the results of inter-laboratory testing. Although there is no standard rule, the acceptance criterion is often set at about twice the observed between-laboratory standard deviation. In this case, the between laboratory precision was very good (an average of about 5%), indicating that a suitable acceptance criterion for other laboratories would likely be no larger than about 10%.

This page left blank intentionally.

5.0 SITE DESCRIPTION

The site selected for the technology demonstration is Operable Unit 1 (OU1) of the Hill Air Force Base (AFB). Detailed information about the site is provided in CH2M HILL (2011). Relevant information for the purposes of this report is summarized in the sections below.

5.1 SITE LOCATION AND HISTORY

Hill AFB is located about 10 miles south of Ogden, Utah. Historically, operations at the base included the use of numerous chemicals, metals, degreasing solvents, and hydrocarbon fuel products that were disposed of in on-base pits and landfills, resulting in soil and groundwater contamination. OU1 of the base is located along the east side of the site and includes several landfills, chemical disposal pits, and fire training areas.

5.2 SITE GEOLOGY AND HYDROGEOLOGY

Geochemically reduced shallow groundwater within OU1 has resulted in the mobilization of naturally occurring metals (including iron, manganese, and arsenic) from soil into groundwater. Metals dissolved in the shallow on-base groundwater have been transported to several hillside springs and seeps immediately north of the OU1 source area. Once exposed to air, the metals have precipitated in the soils near the springs and seeps.

5.3 CONTAMINANT DISTRIBUTION

One such spring (designated U1-305) is referred to as "Site 2". Currently, the arsenic-contaminated sediment at Site 2 appears as stained surface soil along the steep hill slope. Sampling at Site 2 was conducted in August, 2009. In order to achieve spatial representativeness, samples were collected from three separate zones along the more contaminated dry channel that extends downhill from U1-305. Four sediment samples were collected from each of the three zones. Arsenic concentrations detected in these 12 soil samples ranged from 105 to 458 milligrams per kilogram (mg/kg).

Following receipt of the total arsenic results, six of the 12 collected sediment samples were selected for arsenic IVBA testing and arsenic speciation. Before IVBA analysis, these samples were sieved to yield the fine fraction (particles < 250 micromoles [μm]). The following table summarizes the total arsenic concentration measured in each of the six sieved samples selected for IVBA analysis:

Sample ID	Arsenic Concentration (mg/kg)
U1-5212	205
U1-5213	138
U1-5216	192
U1-5218	137
U1-5221	118
U1-5223	172

This page left blank intentionally.

6.0 TEST DESIGN

6.1 CONCEPTUAL EXPERIMENTAL DESIGN

The technology demonstration at the Hill AFB consists of measuring the IVBA of arsenic in several sediment samples collected from Site 2 of OU1, using the measured IVBA values to predict the site-specific RBA of arsenic in these samples, and then comparing the estimated human health risks using the default RBA and the site-specific RBA.

6.2 BASELINE CHARACTERIZATION

In the absence of site-specific data, the national default (baseline) assumption used in the risk assessment is that the RBA of arsenic in soil and sediment is 100%.

6.3 TREATABILITY OR LABORATORY STUDY RESULTS

No treatability studies were performed as part of this project.

6.4 FIELD TESTING

No field testing was performed as part of this project.

6.5 SAMPLING METHODS

Sediment samples from Site 2 of OU1 were collected using standard field collection techniques (see CH2M HILL, 2011).

6.6 SAMPLING RESULTS

6.6.1 IVBA and Speciation Results

Arsenic IVBA testing and arsenic speciation of the six selected samples was performed by the Laboratory for Environmental and Geological Studies at the University of Colorado, Boulder. Detailed information on the arsenic IVBA procedures and speciation methodology utilized to evaluate these samples is provided in CH2M HILL (2011). In brief, arsenic IVBA was determined based on pH 1.5 extraction fluid conditions in accordance with the standard extraction procedure. IVBA results are summarized below:

Sample ID	IVBA (pH 1.5)
U1-5212	13%
U1-5213	5%
U1-5216	18%
U1-5218	17%
U1-5221	28%
U1-5223	12%

Arsenic speciation was performed using an electron microprobe. The results were expressed as the length-weighted frequency and as the relative arsenic mass in a variety of identified arsenic-

bearing phases. Nearly all of the identifiable arsenic in the samples was associated with iron as iron oxide/hydroxide (FeOOH).

6.6.2 RBA Prediction

At the time of the human health risk assessment (HHRA), the regression models developed during this demonstration project had not yet been completed. Rather, CH2M HILL (2011) estimated RBA for arsenic utilizing a regression model that that was based on a set of eight soil samples where the predominant form of arsenic was FeOOH. The resulting best-fit model was:

$$RBA = 14.465 + 0.159 \cdot IVBA(pH\ 1.5)$$

Based on this prediction model, site-specific RBA values for arsenic ranged from 15% to 19%, with a median (and mean) value of 17%. If RBA values were predicted utilizing the recommended model identified in this project, the predicted site-specific RBA values for arsenic would have ranged from 23% to 37%, with a median (and mean) value of 29%.

6.6.3 Impact on Risk

In the HHRA, risks from arsenic in Site 2 were evaluated for two receptor populations (hypothetical residents and visitors/trespassers) based on particulate inhalation and ingestion exposure scenarios. The following table illustrates how the estimated cancer risks from ingestion exposures to arsenic in sediment at Site 2 differ depending upon the selected arsenic RBA value:

Receptor	Cancer Risk Estimates		
	National default RBA of 100%	Site-specific predicted RBA of 17%[a]	Site-specific predicted RBA of 29%[b]
Hypothetical resident	4E-04	7E-05	1E-04
Visitor/trespasser	6E-06	1E-06	2E-06

[a]Based on FeOOH model provided in Attachment D of CH2M HILL (2011)
[b]Based on model provided in Phase IV Report

As seen, compared to the default, use of site-specific RBA values derived from pH 1.5 IVBA measurements resulted in a decrease of risk estimates from above EPA's typical level of concern (>1E-04) to within EPA's typical risk range (1E-04 to 1E-06), such that remedial actions would not be needed.

7.0 PERFORMANCE ASSESSMENT

The performance of the IVBA approach for estimation of RBA of a specific soil sample cannot be evaluated without performing an independent *in vivo* study of RBA on the same test soil. However, based on the regression model for swine data, it is expected that an RBA value estimated from IVBA is likely to be accurate within about 10% of the value that would have been obtained by measurement *in vivo*.

This page left blank intentionally.

8.0 COST ASSESSMENT

8.1 COST MODEL

Table 3 summarizes the cost elements in obtaining an IVBA value needed to calculate an RBA value for a sample of soil or sediment.

Table 3. Cost Model for Conducting an IVBA Test for Estimating the RBA of Arsenic from Soil

Cost Element	Unit Cost
Collect samples for analysis	$200-$300[a]
Dry and sieve samples to isolate the fine-grained fraction	$15-$25
Analyze fine-grained samples for total arsenic[b]	$20-$30
Conduct IVBA Assay (extract sample, measure arsenic in extraction fluid)	$75-$125[c]

[a]Cost not tracked as part of this demonstration, typical range is provided
[b]Sample digestion by EPA Method 3050 followed by sample analysis by EPA Method 6020
[c]Cost per sample usually decreases as sample number increases

8.1.1 Cost Element: Collect and Prepare Soil Samples

The cost of sample collection and preparation was not tracked in this demonstration. Samples may be collected using traditional field sampling techniques and may be either grab samples or composites (the latter is generally preferred for risk assessment purposes). Costs of sample collection vary widely from site to site, but are often in the $200-$300 per sample range.

Once collected, the samples are shipped to a laboratory for preparation and analysis. Preparation steps include drying, mixing and (usually) sieving to a particle size of $\leq$250 µm, since this is the particle size that is generally believed to be of greatest concern for ingestion by the hand-to-mouth exposure pathway. Costs of drying and sieving are typically about $15-25 per sample.

8.1.2 Cost Element: Analyze Soil Samples for Total Arsenic

Once the sample is dried and sieved, the sample is digested according to EPA Method 3050, followed by analysis according to EPA Method 6020. The total cost of digestion and analysis varies from laboratory to laboratory, but typical costs are about $20-$30 per analysis. Duplicate analyses of IVBA test materials are generally recommended to help ensure reliable calculations.

8.1.3 Cost Element: Conduct IVBA Assay(s)

Each soil requires extraction in one or more extraction fluids. Assuming the goal is to predict RBA as measured in the swine bioassay, extraction in pH 1.5 fluid is recommended. In general, each sample should be extracted in duplicate to help ensure the IVBA value is precise.

Several commercial laboratories currently offer the IVBA extraction assay. The cost of the analysis varies between laboratories, but the following breakdown is representative:

- Setup $300-$500
- IVBA Extraction $30-$50 per extraction
- Fluid analysis $30-$50 per analysis

Because of the setup cost, there is generally an economy of scale, with decreased cost per sample as the number of samples increases. For a site where 20 test soils were collected, the total cost would be about $1,500-$2,500 for singlicate analysis (about $75-$125 per sample), and $2,700-$4,500 for duplicate analysis (about $135-$225 per sample).

8.2 COST DRIVERS

The principal cost driver for this technology is the number of independent samples needed to adequately characterize the IVBA of arsenic at a site. If available site information suggests the site is likely to be relatively homogeneous with respect to arsenic mineralogy and soil chemistry, then a relatively small number of samples (e.g., four to six) may be sufficient to derive a reliable and robust estimate of RBA. However, if available site information suggests the site is likely to be relatively heterogeneous (e.g., differing types of mine waste and/or differing soil types at different locations across the site), then it may be necessary to collect and analyze a larger number of samples (e.g., 20-40 or possibly more) to obtain reliable and representative information.

8.3 COST ANALYSIS

The total cost of implementing an IVBA-based approach for estimation of site-specific RBA values for arsenic is provided in Table 4, along with a comparison to the cost of obtaining RBA measures using an *in vivo* animal study. The site where the approach is implemented is assumed to be heterogeneous in arsenic types that are present, such that a total of 20 samples are required to achieve good spatial coverage.

Table 4. Cost analysis for conducting an IVBA study at a heterogeneous site (N = 20).

Cost Element	IVBA		*In Vivo*	
	Unit Cost	**Total Cost**	**Unit Cost**	**Total Cost**
Collect samples for analysis	$200-$300	$4,000-$6,000	$200-$300	$4,000-$6,000
Dry/sieve samples for analysis	$15-$25	$300-$500	$15-$25	$300-$500
Analyze soil samples for arsenic	$20-$30	$800-$1,200	$20-$30	$800-$1,200
Measure IVBA or RBA	$75-$125	$1,500-$2,500	$40,000-$60,000	$800,000-$1,200,000
Total Cost		**$6,200-$9,600**		**$804,700-$1,207,100**

As shown, the total cost of estimating RBA for 20 samples using the IVBA method is less than $10,000, while the cost of obtaining the same data via *in vivo* studies may exceed $1,000,000. In addition, the IVBA studies could be completed within weeks, while the *in vivo* studies would likely require a year or more to complete.

9.0 IMPLEMENTATION ISSUES

9.1 REGULATORY ACCEPTANCE

As noted above, the EPA and other regulatory agencies generally accept and support the concept of incorporating reliable RBA data into site-specific risk assessments (e.g., see http://www.epa.gov/superfund/health/contaminants/bioavailability/bio_guidance.pdf), but do not automatically accept an *in vitro*-based approach for estimation of RBA.

In order to maximize the probability of regulatory acceptance of the IVBA-based method for arsenic, this project has been performed using an approach similar to the approach that was previously followed to develop and gain regulatory acceptance for an IVBA-based method for estimating the RBA of lead in soil (EPA, 2007a). This approach involves frequent presentations to and discussions with EPA's Bioavailability Subcommittee of the TRW to ensure they accept the approach that is being developed and to incorporate any recommendations they may offer, as well as consideration of the guidelines for acceptance of *in vitro* methods described in EPA (2007a). An arsenic *in vitro* method validation assessment report (Griffin, 2012) has been prepared and submitted to EPA to document that the method meets all specified method validation criteria and regulatory acceptance criteria for *in vitro* methods specified in the bioavailability guidance (EPA, 2007a).

In accord with this approach, from 2009 through 2012, Dr. Griffin has attended several meetings of the TRW subcommittee to present the current progress and findings of the project. We have also been in discussions with the co-chairs of this subcommittee on bringing the method development, validation, and SOPs to the TRW for national acceptance. National acceptance of the arsenic *in vitro* methodology developed through the Environmental Security Technology Certification Program (ESTCP) grant should remove all federal and state regulatory barriers to the use of IVBA tests to adjust bioavailability factors in risk assessment equations and cleanup level development.

9.2 PROCUREMENT OF IVBA ANALYSES

Although IVBA extractions are not a routine service provided by all analytical laboratories, there are several laboratories that currently have the equipment and provide the services. This includes both commercial laboratories as well as several EPA regional laboratories.

This page left blank intentionally.

10.0 REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR). 2007. Toxicological Profile for Arsenic. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. August 2007.

Basta, N.T., J.N. Foster, E.A. Dayton, R.R. Rodriguez, and S.W. Casteel. 2007. The Effect of Dosing Vehicle on Arsenic Bioaccessibility in Smelter-Contaminated Soils. J. Envir. Science and Health. Part A., 42, pp. 1275-1281.

Bruce, S., B. Noller, V. Matanitobua, and J. Ng. 2007. In Vitro Physiologically Based Extraction Test (PBET) and Bioaccessibility of Arsenic and Lead from Various Mine Waste Materials. J. Tox. and Envir. Health. Part A., pp. 1700-1711.

Buckley, B.T. 1997. Estimates of Bioavailability of Metals in Soil with Synthetic Biofluids: Is This a Replacement for Animal Studies? In: IBC Conference on Bioavailability. 1998, Scottsdale, Arizona, USA.

CB Research International (CBR). 1993. Report: Development of a Physiologically Relevant Extraction Procedure. Sidney, British Columbia, Canada.

Drexler, J.W. 1998. An In Vitro Method That Works! A Simple, Rapid, and Accurate Method for Determination of lead Bioavailability. EPA Workshop, Durham, North Carolina.

Drexler, J.W. 1997. Validation of an In Vitro method: A tandem approach to Estimating the Bioavailability of Lead and Arsenic in Humans. IBC Conference on Bioavailability. Scottsdale, Arizona.

Drexler, J.W. 2000. Bioavailability/bioaccessibility of metals. Paper read at The ITRC Fall 2000 Conf.: New Environ. Technol. and Market Opportunities, at San Antonio, Texas. 16–20 Oct.

Drexler, J., and W. Brattin. 2007. An In Vitro Procedure for Estimation of Lead Relative Bioavailability: With Validation. Human and Ecological Risk Assessment. 13(2), pp. 383-401.

Ellickson, K.M., R.J. Meeker, M.A. Gallo, B.T. Buckley, and P.J. Lioy. 2001. Oral Bioavailability of Lead and Arsenic from a NIST Standard Reference Soil Material., Arch. Environ. Contam. Toxicol., 40, pp. 128-135.

Griffin, S. 2012. Validation Assessment of *In Vitro* Arsenic Bioaccessibility Assay for Predicting Relative Bioavailability of Arsenic in Soils and Soil-like Materials at Superfund Sites. November 2012.

Juhasz, A.L., E. Smith, J. Weber, M. Rees, A. Rofe, T. Kuchel, L. Sansom, and R. Ravi Naidu. 2006. In Vivo Assessment of Arsenic Bioavailability in Rice and Its Significance for Human Health Risk Assessment. Environ. Health Perspect. 114(12): 1826-1831.

Juhasz, A.L., E. Smith, J. Weber, M. Rees, A. Rofe, T. Kuchel, L. Sansom, and R. Naidu. 2007. In Vitro Assessment of Arsenic Bioaccessibility in Contaminated (Anthropogenic and Geogenic) Soils. Chemosphere 69: 69–78.

Malinowski, H., P. Marroum, V.R. Uppoor, et al. 1997. Draft guidance for industry extended release solid oral dosage forms. In: Young D, Devane J, and Butler J (eds), Vitro-in Vivo Correlations. Plenum Press, New York, NY, USA

Medlin, E.A. 1997. An In Vitro Method for Estimating the Relative Bioavailability of Lead in Humans. Master's thesis. Department of Geological Sciences, University of Colorado, Boulder.

Oomen, A.G., A. Hack, M. Minekus, E. Zeijdner, C. Cornelis, G. Schoeters, W. Verstraete, T. Van de Weile, J. Wragg, C.J.M. Rompelberg, A.J.A.M. Sips, and J. Van Wijnen. 2002. Comparison of Five In Vitro Digestion Models to study the Bioaccessibility of Soil Contaminants. Envirn. Sci Tech. 36: 3326-3334.

Roberts, S.M., J.W. Munson, Y.W. Lowney, and M.V. Ruby. 2007. Relative Oral Bioavailability of Arsenic from Contaminated Soils Measured in the Cynomolgus Monkey. Toxicological Sciences 95:281-288.

Rodriguez, R.R., N.T. Basta, S.W. Casteel, and L.W. Pace. 1999. An In Vitro Gastrointestinal Method to Estimate Bioavailable Arsenic in Contaminated Soils and Solid Media. Environ. Sci. Technol. 33, 642-649.

Ruby, M.W., A. Davis, R. Schoof, S. Eberle, and C.M. Sellstone. 1996. Estimation of Lead and Arsenic Bioavailability Using a Physiologically Based Extraction Test. Environ. Sci. Technol. 30(2):422–430.

U.S. Environmental Protection Agency (EPA). 1989. Risk Assessment Guidance for Superfund Volume I Human Health Evaluation Manual. Office of Emergency and Remedial Response, U.S. Environmental Protection Agency, Washington D.C. EPA/540/1-89/002.

EPA. 2007a. Guidance for Evaluating the Oral Bioavailability of Metals in Soils for Use in Human Health Risk Assessment. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response. Washington, DC 20460. OSWER 9285.7-80. Available online at: http://www.epa.gov/superfund/health/contaminants/bioavailability/bio_guidance.pdf.

EPA. 2007b. Framework for Metals Risk Assessment. Office of the Science Advisor Risk Assessment Forum. EPA/120/R-07/001.

EPA. 2010. Relative Bioavailability of Arsenic in Soils at 11 Hazardous Waste Sites Using an In Vivo Juvenile Swine Method. Bioavailability Subcommittee of the Technical Review Workgroup, Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, DC. OSWER Directive #9200.0-76. June 2010. Available online at: http://epa.gov/superfund/bioavailability/pdfs/as_in_vivo_rba_main.pdf.

APPENDIX A

POINTS OF CONTACT

Point of Contact	Organization	Phone Fax E-Mail	Role In Project
Susan Griffin	EPA, Region 8 15195 Wynkoop Street Denver, CO 80202	Phone: 303-312-6651 E-mail: griffin.susan@epa.gov	Co-investigator
Yvette Lowney	Exponent, Inc. 4141 Arapahoe Avenue Suite 101 Boulder, CO 80303	Phone: 303245-7070 E-mail: ylowney@exponent.com	Co-investigator
John Drexler	University of Colorado Dept. of Geological Sciences Benson Earth Science Bldg. 2200 Colorado Avenue Boulder, CO 80309	Phone: 303492-5251 E-mail: drexlerj@colorado.edu	Laboratory Director
William Brattin	SRC, Inc. 999 18th Street, Suite 1150 Denver CO 80202	Phone: 303-357-3121 E-mail: brattin@srcinc.com	Contributing Scientist
Gary Diamond	SRC, Inc. 8191 Cedar Street Akron, NY 14001	Phone: 716-542-7140 E-mail: diamond@srcinc.com	Contributing Scientist
Lynn Woodbury	CDM Smith 555 17th Street, Suite 1100 Denver, CO 80202	Phone: 303-383-2382 Email: woodburyL@cdmsmith.com	Contributing Scientist
Andrea Leeson	ESTCP 4800 Mark Center Drive Suite 17008 Alexandria, VA 22350-3605	Phone: 571-372-6398 E-mail: andrea.leeson@osd.mil	Environmental Restoration Program Manager

ESTCP Office
4800 Mark Center Drive
Suite 17D08
Alexandria, VA 22350-3605
(571) 372-6565 (Phone)
E-mail: estcp@estcp.org
www.serdp-estcp.org